# Toroidal proteins: **Running rings around DNA**

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**Recent structural data indicate that the toroidal form is quite common among DNA-binding enzymes. Is this abundance of ring-shaped proteins a coincidence, or does it reflect convergence to a winning quaternary structure?**

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In recent years there has been a veritable explosion of information on the structure and mechanism of enzymes involved in DNA metabolic pathways. This explosion reflects the fundamental importance of processes such as DNA replication for the propagation of life, as well as the incredible variety of enzymatic activity that makes DNA metabolism a fascinating area for research. Proteins involved in DNA metabolism range from enzymes that synthesize DNA to enzymes that degrade DNA, with enzymes that catalyse all kinds of DNA manipulations in between.

The study of this bewildering array of enzymes is aided considerably by their conservation through evolution, whereby important structural motifs are relatively untouched by evolutionary change. It is common to find that proteins performing the same task in widely divergent organisms have similar sequences and structures. A well-known example of such conservation is the DNA/RNA polymerase family, members of which have been described as "variations on a single theme" [1]. *Escherichia coli* DNA polymerase I Klenow fragment, bacteriophage T7 RNA polymerase and HIV-1 reverse transcriptase, among other polymerases, exhibit common features that range from sequence identity between catalytically important amino acids to the overall 'hand'-shaped arrangement of structural domains.

This similarity between enzymes — often inferred to be indicative of true evolutionary homology — is used routinely by molecular biologists to facilitate the elucidation of enzyme structure and mechanism. But while attention has focused on shared features of functionally related enzymes, relatively little notice has been given to the emerging pattern of structural similarity among functionally unrelated enzymes. A striking illustration of this theme is the toroidal form adopted by proteins that perform very diverse functions in DNA metabolism (Figure 1). So far, the toroidal form has been found in processivity factors, DNA replication initiators, helicases, transcription terminators and a DNA-binding protease. The most recent addition to this group of enzymes is λ exonuclease, a trimeric ring that degrades one strand of duplex DNA during homologous recombination [2].

## Ring leaders

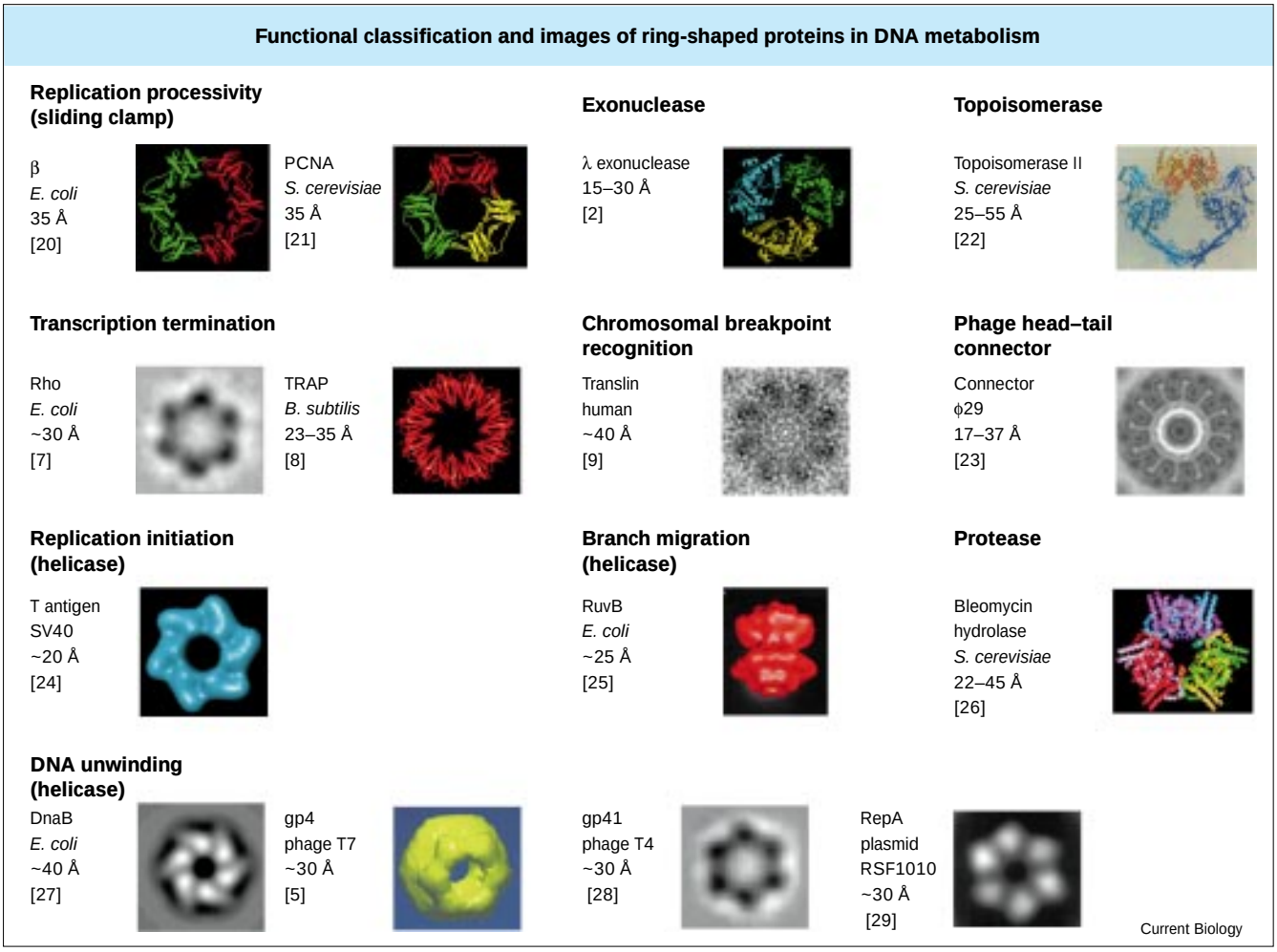
Ring-shaped enzymes involved in DNA metabolism vary greatly in sequence, structure and function, but for this discussion they have been divided into two groups — proteins that bind DNA and proteins that are catalytically active on DNA. Among the DNA-binding toroids are 'sliding clamps' that encircle DNA and serve as processivity factors for other proteins — that is, they help other proteins stay bound to DNA through multiple catalytic turnovers. Thus, bacteriophage T4 gp45, *E. coli* β factor, and eukaryotic PCNA ('proliferating cell nuclear antigen') all tether their respective DNA polymerase partner to template DNA, allowing it to replicate several thousand bases without dissociation [3].

Although they are best known for their role in DNA polymerase processivity, sliding clamps may serve other DNA metabolizing enzymes as well. PCNA also binds the 'flap' endonuclease FEN-1, the repair exo/endonuclease XPG and DNA-(cytosine-5) methyltransferase; like DNA polymerase these enzymes may also piggyback on PCNA for efficient translocation to sites of activity on DNA. In sliding clamps, the two essential requirements for processivity, stable interaction with DNA as well as freedom of movement on DNA, have been met by formation of a topological link between a protein ring and DNA.

Other DNA-binding toroids are catalytically active on DNA. Hexameric helicases, such as *E. coli* DnaB, bacteriophage T7 gp4 and bacteriophage T4 gp41, catalyze unwinding of duplex DNA to generate single-stranded templates for DNA replication, recombination and repair [4]. The helicase ring encircles one DNA strand at the fork junction and probably unwinds DNA by translocating on that strand and excluding the other strand from the central channel [5,6]. *E. coli* Rho is also a ring-shaped hexamer, but it functions as a transcription terminator by unwinding RNA:DNA duplexes and releasing nascent RNA transcripts from elongation complexes [7].

Bacteriophage λ exonuclease catalyses processive degradation of one strand of duplex DNA in the 5′→3′ direction to generate single-stranded DNA for homologous DNA recombination. The crystal structure of λ exonuclease, solved recently by Kovall and Matthews [2], shows three

**Figure 1**




Functional classification and images of ring-shaped proteins in DNA metabolism. The structural graphics show top views of the rings, and channel diameters are indicated.

exonuclease subunits in a circular assembly that is large enough to accommodate double-stranded DNA at one end and single-stranded DNA at the other. This information has provided a structural basis for the processive activity of λ exonuclease: the enzyme may take double-stranded DNA into the central channel at one end of the ring, degrade one strand, and extrude single-stranded DNA from the other end, effectively translocating on DNA for continuous hydrolysis of several thousand bases [2].

Other enzymes in DNA metabolism are known to form rings, but their potential interactions with DNA have not been studied. The *Bacillus subtilis trp* RNA-binding attenuation protein (TRAP), for example, forms an ondecamer ring that acts as a transcription terminator. Specific binding sequences on nascent RNA wrap around the TRAP ring to form a terminator structure that signals the end of transcription [8]. It has been suggested that TRAP may also use its ring shape to translocate on DNA (or RNA), close to RNA polymerase, until it reaches its target binding sites on the transcript, but this idea has not yet been tested.

Another protein, Translin, forms an octamer ring that binds single-stranded DNA ends at chromosomal breakpoints [9]. There is no evidence for topological linkage between Translin and DNA, but one use for the ring shape may be to allow Translin to track on DNA until it reaches a staggered breakpoint. In fact, topological linkage between a protein ring and DNA has been demonstrated only for sliding clamps [10], some hexameric helicases [4] and the bovine papillomavirus E1 replication initiator [11], but all known ring-shaped enzymes in DNA metabolic pathways have a central channel large enough to accommodate single-stranded or double-stranded DNA (Figure 1).

**If the ring fits**...

The toroidal form is not uncommon in nature. Prominent examples of ring-shaped proteins include molecular chaperones such as GroEL, cell cycle regulatory proteins such as CksHs2, proteasomes, bacterial light-harvesting complexes such as LH1 and LH2, and $F_1$-ATPase, the catalytic component of ATP synthase. A circular protein provides an enclosed environment for catalytic activity — a necessity for proteolytic degradation catalyzed by proteasomes [12]. The oligomeric assembly provides a distinctive array of identical ligand-binding sites; for example, in CksHs2, binding of cyclin-dependent kinase monomers at sites inside the ring may lead to their oligomerization and activation [13]. A ring can also be used to drive conformational changes of macromolecules located in the central channel, as seen in chaperones [14] and in $F_1$-ATPase [15].

Ring-shaped proteins in DNA metabolism form a distinctive subset of the toroid family. While toroids are used for a variety of functions in the cell, it is noteworthy that in DNA metabolism almost all classes of enzymes have a ring-shaped representative or employ one as part of a functional complex (Figure 1). These enzymes belong to different protein families on the basis of their catalytic activity and also have different postulated primordial progenitors. Thus, a helicase is a nucleotide-triphosphate-driven motor protein and λ exonuclease is a nucleotidyl hydrolase, but both assemble into multisubunit rings. Why have so many enzymes adopted the same shape? One reason may be that, although functionally diverse, these enzymes share DNA as a substrate, and the distinctive structure and properties of DNA have facilitated widespread convergence to the ring shape.

In addition to the advantages noted earlier, a circular assembly offers unique benefits to DNA-binding proteins, the most important being the ability to form a topological link with DNA. This linkage has conferred processivity on hexameric helicases, sliding clamps (DNA polymerase), λ exonuclease and possibly other proteins as well. Consequently, these enzymes have acquired the speed necessary for the manipulation of long chromosomal DNA within the time frame of one cell cycle. Given the importance of processivity in DNA replication, repair and recombination, it is likely that the toroidal form has had significant impact on the evolution of DNA metabolism to its current level of efficiency.

The formation of a topological link between protein and DNA does not necessarily require multisubunit assembly. DNA-binding toroids are oligomers probably because the construction of a circular structure by multiplication and assembly of subunits is simpler than the design of one large protein with a central cavity. Oligomeric assembly also has the advantage of generating multiple identical binding sites on the ring around the DNA. In some enzymes, this circular arrangement of catalytic sites may be fundamental to their enzymatic activity; for example, there is some evidence that subunits of a helicase hexamer may bind and release DNA sequentially to promote translocation of the ring on DNA [16,17]. In this case, the arrangement of subunits probably facilitates rapid cycling of the encircled DNA between active sites, and the ring shape ensures that, on release from one site, the DNA does not dissociate from the hexamer. λ exonuclease also has three sites that may surround DNA, but it is not known whether all three must be catalytically active for nuclease activity. It would be interesting to determine whether the arrangement of three subunits around DNA confers mechanistic benefits other than processivity on λ exonuclease.

DNA binding within the central channel requires a mechanism to allow DNA access to binding sites on the protein. Studies on toroidal DNA-binding proteins so far indicate that the protein ring opens to allow DNA entry into the channel. Sliding clamps are opened and assembled around DNA by complex machines known as 'clamp-loaders', whereas helicase rings can self-assemble around DNA. The requirement for ring opening may allow toroidal proteins to discriminate between binding to DNA ends and binding to internal sites on DNA. It has been demonstrated that λ exonuclease cannot digest closed circular DNA substrates [18]. If the λ exonuclease ring is stable — that is, cannot open or assemble around DNA — and if it does not have an accessory 'exonuclease-loader', then it can only bind DNA by sliding onto ends. This property would preclude the enzyme from digesting DNA at internal sites, and would serve to target λ exonuclease activity specifically to DNA ends, such as those that occur in the double-strand break repair pathway of DNA recombination. Similarly, if the Translin ring cannot assemble around DNA, it may bind DNA only by sliding on at the ends and thus specifically target chromosomal breakpoints. Other proteins that bind DNA only at ends, such as Ku antigen, may also have a toroidal form [19].

The ring shape may also be used to distinguish between binding to single-stranded DNA or double-stranded DNA. Sliding clamps do not have specific DNA-binding sites, but the circular assembly creates a positively charged channel in which duplex DNA can bind and slide freely. The ring may not interact with single-stranded DNA in the same fashion because of the asymmetry of negative charge on single-stranded DNA. This property may be important during DNA replication by helping to direct clamp loading specifically to primed sites on the single-stranded DNA template.

In contrast to double-stranded-DNA-binding clamps, the hexameric helicase T7 gp4 binds single-stranded DNA preferentially over double-stranded DNA. This ability to

bind one single-stranded DNA strand and exclude double-stranded DNA from the central channel facilitates DNA unwinding. λ exonuclease is an interesting case in which the toroidal form may direct exclusive binding to single-stranded DNA and double-stranded DNA all within one molecule. If duplex DNA fits into only one end of the ring and single-stranded DNA in the other, as suggested, then in addition to processivity, the ring shape is also responsible for directional activity of λ exonuclease on double-stranded DNA. It appears that use of the toroidal form for DNA binding has promoted the development of proteins with highly specialized structural features and novel mechanisms of enzymatic activity.

### In closure

Nature seems to have reinvented the wheel for a number of different biological functions. In particular, the toroidal form is used liberally in DNA metabolic pathways, and we have argued that this may be due to special advantages of binding DNA by circular assembly. Given the rapid advances in technology for visualization of macromolecules, it is highly likely that even more ring-shaped proteins will be discovered. For us, there is ample evidence already that the toroid is a remarkable quaternary structure and it will be hard to find a more elegant and universally effective design of biological machinery.

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